Use of In Vitro Studies To Characterize Relative Toxicity of LA

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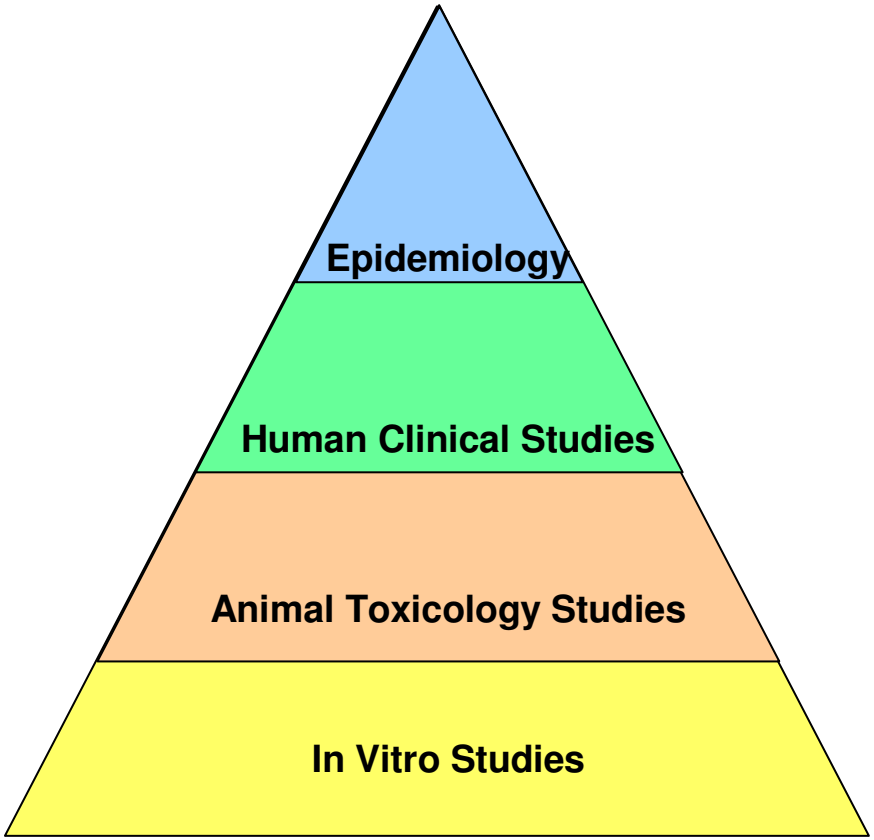
Libby OU4 Meeting
September 26, 2007

In Vitro Toxicology

- Useful for rapid screening of complex mixtures to identify components responsible for causing adverse health effects
- Identify mechanisms by which air pollutants damage lung cells
- Focuses on relevant target cell types
- May be difficult to predict human responses due to limitations in methodology

In Vitro Toxicology

Because of rapid screening ability, in vitro studies can serve as a foundation for costlier and more complex animal toxicology studies, human clinical studies and epidemiological studies



In Vitro Toxicology

Cellular toxicity caused by several kinds of asbestos fibers can be quickly determined

Goal: Compare the ability of asbestos obtained from several sources to cause significant biological effects in cultured cells

How Realistic Are In Vitro Experiments?

- Cells removed from their normal 3 dimensional environment.
- No blood supply with potentially important factors.
- Exposure not likely the same as in vivo.

However

- Many cellular functions are still active
- Appropriate cell types can be chosen and a realistic dose response curve generated

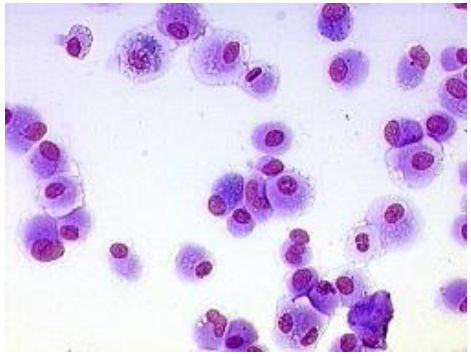
Role of In Vitro Experiments to Characterize Relative Toxicity of LA

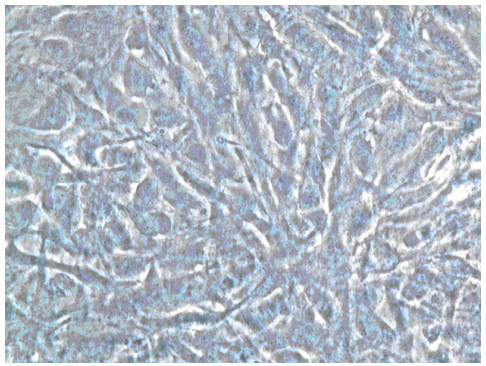
- Oxidative Stress: a mechanism by which asbestos causes initial cellular injury. Different types of asbestos have lesser or greater capability of inducing oxidative stress.
- Compare the response of macrophages and epithelial cells to oxidative stress induced by LA and other fibers/particles: assemble relative toxicity chart predictive of acute pulmonary injury in rodents and humans.
- Combination of severity of oxidative stress, biopersistence, and kinetics of distribution will ultimately determine the chronic effects cause by LA.

Ensure Relevance of In Vitro Studies

- Use appropriate cells

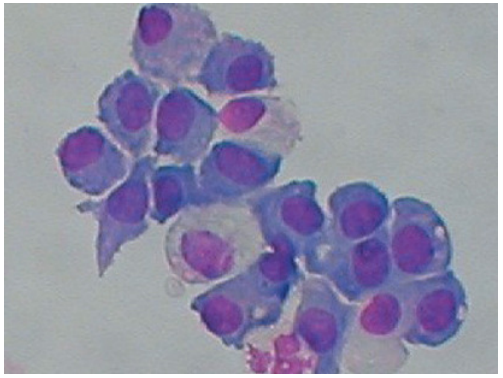
alveolar macrophages

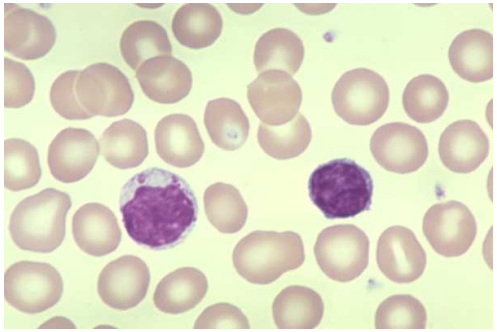




epithelial cells

mesothelial cells





Lymphocytes (with RBCs)

- Use a wide range of doses
- Confirm whenever possible using in vivo approaches

Experimental Approach

- Apply range of doses to different cell types
- At various times after exposure, remove cell culture fluid to analyze for mediators
- Isolate RNA to analyze for changes in expression of mediators

Tiered Approach

- Toxicology Assays
- Cell Function Assays
- Cell Signaling Pathways
- Gene Expression Profiling

Toxicology Assay Measurements

- Death of cells:

  Dying cells release LDH

- Injury of cells:

  Injured cells release cytokines such as TNF-α and IL-8 which promote injury and inflammation

- Oxidative stress:

  Heme oxygenase and other indicators provide an index of this mechanism of injury

- DNA damage:

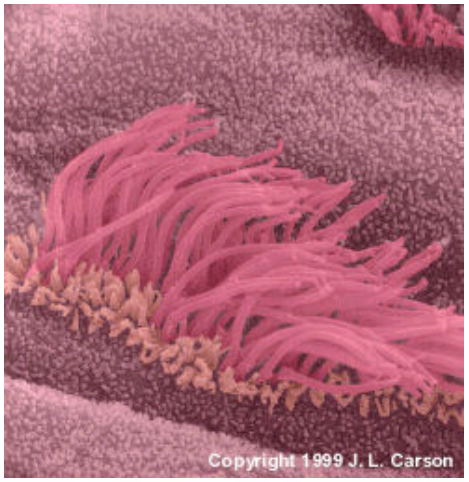
  Comet assay in lymphocytes, mesothelial cells or mesenchymal cells

Cell Function Assays

- Defense against micro-organisms
  - Do fibers inhibit ability of macrophages to
    - engulf bacteria ?
    - produce bacteria-killing reactive oxygen species?

Macrophage engulfing parasite

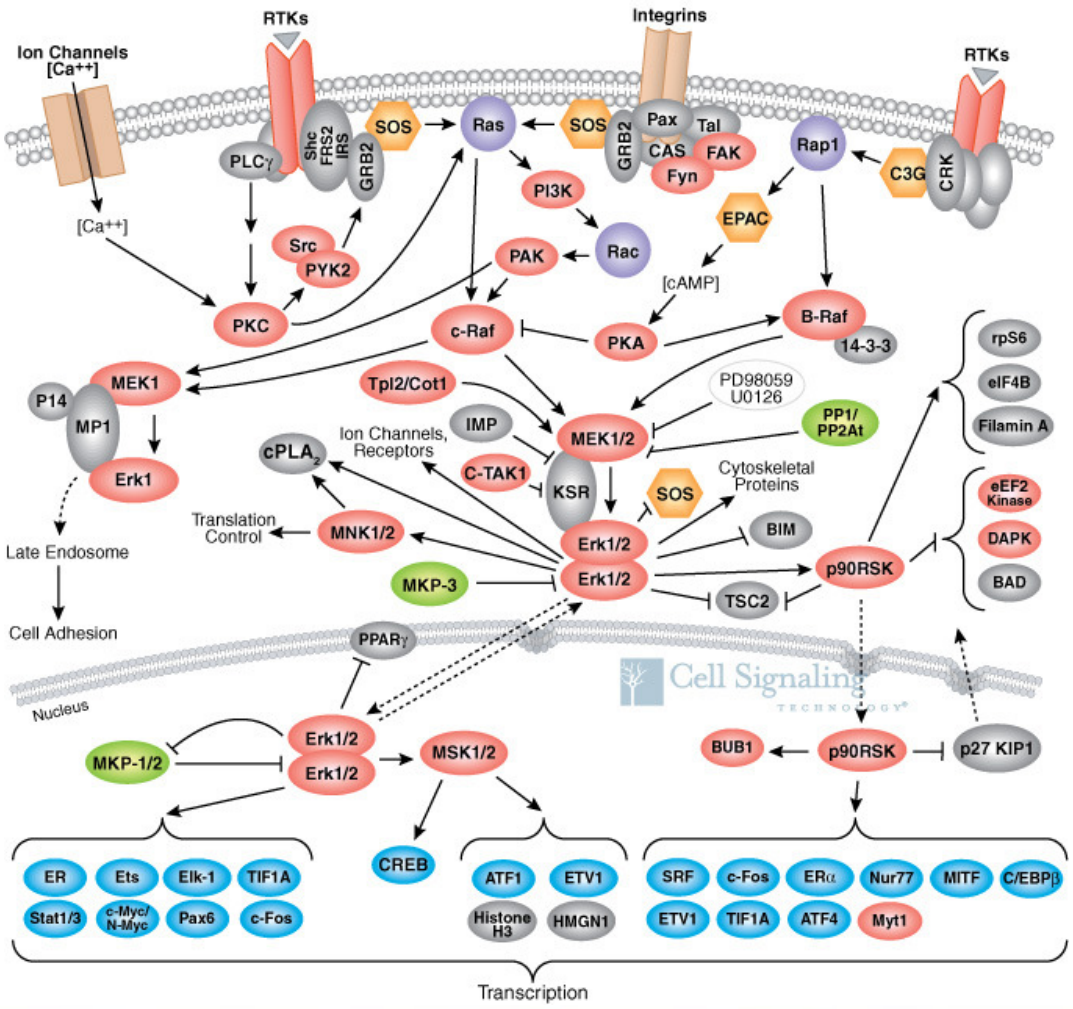
- Defense against inhaled particles
  - Do fibers inhibit epithelial cell
    - production of mucin which protects airways ?
    - function of cilia which moves particles out?
    - tight junctions between cells important for function?



Cilia

Cell Signaling Pathways

- Ability of mineral fibers to activate or repress different signal transduction pathways will be assessed



MAPK/Erk signaling cascade is activated by a wide variety of receptors involved in growth and differentiation

Gene Expression Profiling

- Ability to monitor changes in 40,000 genes simultaneously
- Potential to identify biomarkers of exposure, effect, or sensitivity unique to individual particle types
- Gene changes associated with alterations in specific cell pathways and functions
- most powerful approach to determining the comparative potency of LA when considering numerous samples

Summary

- In vitro assays are very useful for rapid, inexpensive screening of large numbers of samples
- Careful interpretation required to select best candidate samples for further testing in animal studies
- A tiered approach will be used to examine direct cell toxicity, impact on cell function, changes in cell signaling mechanisms, and genes associated with alterations in cell function